REMARKS

In the outstanding Office Action, claims 1 to 36 were presented for examination. Claims 24-35 have been withdrawn from consideration. Claims 1-23 and 36 were rejected on formal grounds under 35 U.S.C. §112. In addition, rejection was advanced variously on the basis of 35 U.S.C. §102 or §103 against claims 1-23 and 36 as being unpatentable over a reference to Wright et al. in view of a reference to inventor Poelstra herein, et al.

The Office Action has been most carefully studied. In this amendment applicant has canceled claims 24-35 *without prejudice*, and has added new claims 37-44 more particularly pointing out the invention. In addition, claims 1-10, 12-23 and 36 have been amended. The new and amended claims have been carefully written to avoid any questions under 35 U.S.C. §112, in accordance with the guidelines and requirements set forth in the outstanding Office Action. Accordingly, as will be discussed in detail below, it is believed that the application is clearly in condition for allowance.

Non-Final Action

In a telephone conversation with applicant's representative, Roger Pitt on November 6, 2002, Examiner Ford kindly confirmed that the outstanding action is non-final as indicated in box 2b of the Office Action Summary, and that section 9 of the Action, which has contrary indications, may be ignored.

Election

For the record, applicant believes that claim 24 has not been fully considered by

the Office, that the claim does in fact recite the limitations of claim 1 by virtue of its dependency therefrom and that claim 24 might properly have been examined with claims 1-23 and 36. Nevertheless, applicant has canceled withdrawn claims 24-35, without prejudice, to expedite allowance.

Claim Rejections - 35 U.S.C. §112 Indefiniteness

Minor amendments have been made to overcome the rejections, without, it is believed, narrowing any claim, by removing minor informalities or making explicit matter which was implicit prior to amendment.

The phrase "the degree of occupancy of lipopolysaccharide binding sites on alkaline phosphatase" refers to the degree of physical association between alkaline phosphatase and LPS. The degree of occupancy is the ratio between the total amount of alkaline phosphatase in the serum or other body fluid sample and the actual alkaline phosphatase activity. This may be understood as the ratio of active to inactive alkaline phosphatase, as is described in the specification for example at page 3, lines 11 to 17 and at page 5, lines 13 to 22 in the original text. The physical association between or binding of LPS with alkaline phosphatase attenuates LPS endotoxicity and decreases alkaline phosphatase enzymatic activity.

Claim Rejections - 35 U.S.C. §103 Unpatentability

Turning now to the rejection of claims 1-23 and 36 as unpatentable over Wright et al. in view of Poelstra *et al. (American Journal of Pathology)*, such rejection is believed not remotely relevant to claims 1-23 and 36 as amended.

Amended claim 1 relates to a method of diagnosis of onset of endotoxemia or sepsis attributable to Gram negative bacterial infection. The method comprises monitoring the degree of occupancy of lipoplysaccharide ("LPS") binding sites on alkaline phosphatase in a sample of fluid derived from a patient. Pursuant to the invention, as explained in the specification and described hereinbelow, it has been discovered that the degree of occupancy in serum is associated with the presence or absence of Gram negative bacterial infection.

The invention as now claimed in amended claim 1 is based on the discovery that the response to LPS in serum is for alkaline phosphatase activity initially to decrease. Only subsequently, after the initial decrease, does the alkaline phosphatase level increase, after LPS has induced liver damage, or has otherwise induced alkaline phosphatase expression. This discovery is neither taught nor remotely suggested by any reference or references of record, or known to applicant, whether considered alone or in combination. New claim 37is directed to a method of diagnosis which is limited to detecting this significant reduction in alkaline phosphatase activity.

While the invention is not limited by any particular theory, it is presently understood by applicant that as LPS reaches toxic levels it induces a slow increase in alkaline phosphatase activity in serum, caused by increased production and/or release

of alkaline phosphatase by the liver. On the other hand, as an initial response, LPS induces a decrease in alkaline phosphatase activity in serum caused by the binding of the LPS with alkaline phosphatase.

Applicant's invention as now claimed in amended base claim 1 for the first time recognizes the existence of these two effects and provides the possibility of discriminating between them by monitoring or measuring the ratio of active to inactive alkaline phosphatase activity in serum to predict, diagnose or monitor a possible Gram-negative endotoxemia. If possible, and desirably, the determination is effected by making a number of determinations over a period of time, for example, as defined in amended claim 3. The ratio or degree of occupancy of alkaline phosphatase, as measured for the diagnostic purposes of the invention, refers to the degree of physical association between alkaline phosphatase and LPS in serum. Such association attenuates LPS endotoxicity and decreases alkaline phosphatase enzymatic activity in serum.

The reference to Wright et al. teaches a method for diagnosis, prognosis and monitoring of gram-negative sepsis, septic shock or endotoxemia by measuring the level of LPS exchange protein activity in a sample. The Wright et al. diagnostic methods comprise:

1) the measurement of the serum concentration of a lipoprotein of an LPS exchange protein as a diagnostic tool for sepsis;
2) the measurement of LPS neutralizing capacity in plasma as a diagnostic tool; and

3) an immunoassay for LPS binding proteins employing labeled antibodies .

Wright et al. neither teach nor suggest that alkaline phosphatase serum levels can be monitored as an indicator of sepsis. Nor, as admitted by the Office, in the first paragraph of page 6 of the Office action, do Wright et al. teach or suggest monitoring the degree of occupancy of LPS binding sites in alkaline phosphatase in a patient serum sample. Nor do Wright et al. suggest that the degree of alkaline phosphatase occupancy is associated with the presence or absence of Gram-negative bacterial infection. In view of these deficiencies, Wright et al. cannot, and does not, suggest the limitation in applicant's new claim 37 of monitoring alkaline phosphatase activity in a to detect a decline of alkaline phosphatase activity indicative of Gram negative bacterial infection.

In contrast, to Wright et al., applicant's claimed diagnostic method for endotoxemia or sepsis does not determine the LPS neutralizing activity *per se* but measures specific alkaline phosphatase levels and enzymatic activity, and can employ a substrate quite unrelated to LPS, pNNP.

Some of the benefits of the claimed diagnostic method over Wright et al.'s measurement of LPS neutralising activity, are illustrated by the following hypothetical examples:

Hypothetical 1. A serum sample with high LPS-neutralizing activity as determined by the Wright et al. assay has a high level of inactive alkaline

phosphatase, the binding sites of which are occupied by LPS. The patient may has a high level of LPS in his serum but may show mild or no symptoms of endotoxemia.

Hypothetical 2. A low serum LPS-neutralizing activity as determined by the Wright assay is accompanied by a low level of inactive alkaline phosphatase. The level of LPS in the serum is low and again the patient may experience mild or no symptoms of endotoxemia.

Applicant's claimed diagnostic method can discriminate between these situations, whereas the method of Wright et al cannot. Although both diagnostic methods are aimed at the diagnosis of endotoxemia, applicant's claimed diagnostic method measures levels of a different target, and in certain significant instances can generate different, more useful results.

The Poelstra et al. (1997) reference does not remotely correct the deficiencies of Wright et al.

The examiner argues that applicant's claimed invention is unpatentable over the a combination of Wright et al. and the teachings of Poelstra et al. (1997). However, such argument is not relevant to applicant's invention as now claimed because the art contains no suggestion to make such a combination and combining these or any other references does not provide applicant's invention.

Poelstra et al. (1997) teaches that alkaline phosphatase activity in tissue is capable of dephosphorylating LPS and that partly dephosphorylated LPS has a reduced toxicity. It is known that LPS causes liver damage, resulting in alkaline phosphatase release into the circulation. Accordingly, alkaline phosphatase is used as a diagnostic marker for infections causing liver damage.

Poelstra et al, (1997) studies alkaline phosphatase activity in tissue and neither teaches nor suggests that alkaline phosphatase activity in serum decreases after LPS exposure of a subject. In light of the present invention it can be understood that alkaline phosphatase activity in tissue does not necessarily correlate with alkaline phosphatase activity in serum.

Furthermore, applicant's invention as now claimed is based upon a first novel discovery that an initial, LPS-induced decrease in serum alkaline phosphatase activity in cases of Gram-negative sepsis. A second novel finding is that the decrease is caused by inhibition of the alkaline phosphatase activity by binding with LPS.

This discovery of the association and inhibition of alkaline phosphatase by LPS in serum is quite distinct from the observation in Poelstra et al. (1997) that LPS can be a substrate for alkaline phosphatase activity in tissue. While reaction product inhibition is a known phenomenon for some enzymes under some conditions, the extremely low, picomolar range of LPS in serum during onset of sepsis make the occupancy and inactivation of circulating alkaline phosphatase by such minuscule concentrations of

LPS an extraordinary observation, neither found nor suggested in any reference of record or known to applicant.

In summary, the occupancy of alkaline phosphatase by LPS and the decrease of alkaline phosphatase activity in serum immediately after administering or exposure to LPS is neither disclosed nor suggested in Poelstra et al (1997), nor in any other reference. Applicant's claimed diagnostic methods based on determination of the ratio of active to inactive alkaline phosphatase in serum and detection of a decline in alkalie phosphatase activity can therefore not remotely be inferred from the art. Accordingly amended base claim 1 and new base claim 37 are believed clearly and patentably distinguished from the cited art or any other art.

Dependent Claims

Claims 2-23 and 36 which depend from amended base claim 1, and new claims 38-44 which depend from new base claim 37 are therefore believed allowable with claims 1 and 37 for the reasons that claims 1 and 37 are allowable. Dependent claims 2-23 and 36 and 38-44 are furthermore clearly and patentably distinguished from the art of record, and therefore allowable, by the additional meaningful limitations they recite.

The remaining art of record has been carefully considered, but does not appear to be remotely relevant to any of applicant's claims.

In view of the above amendments and the discussion relating thereto, it is respectfully submitted that the instant application, as amended, is in condition for

allowance. Such action is most earnestly solicited. If for any reason the Examiner feels that consultation with Applicant's representative would be helpful in the advancement of the prosecution, he is invited to call the telephone number below for an interview.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on March 11, 2003

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